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ACUTE TOXICITY OF AMMONIA TO THE WHITE SUCKER

by

Keturah A. Reinbold
Stephen M. Pescitelli

Illinois Natural History Survey
Aquatic Biology Section
Champaign, Illinois 61820

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INTRODUCTION

Ammonia is an important pollutant in natural waters both as a toxicant and as an oxygen demanding material. It commonly occurs in municipal and industrial waste discharges and in runoff from agricultural feed lots. Aqueous solutions of ammonia are very toxic to fishes under certain environmental conditions, and the toxicity is primarily attributed to the un-ionized form. The percent of total ammonia occurring in the un-ionized form depends upon pH, temperature and, to a lesser degree, ionic strength.

Frequency of occurrence and the high cost of treatment for adequate removal have made the issue of water quality criteria for ammonia very difficult. It is essential to develop a broad data base for ammonia toxicity to fish. Information on toxicity during all life stages is needed to insure protection throughout the entire life-cycle.

Early life stage tests are less costly and time consuming than entire life-cycle tests and may allow a realistic estimate of the maximum allowable concentration of a chemical to a fish species. However, acute toxicity data from tests conducted under conditions similar to early life stage tests are needed in order to determine realistic application factors. Information on the effects of exposure to ammonia on the egg and larval stages are available for white sucker (Reinbold and Pescitelli 1982). However, there are no published reports on the acute toxicity of ammonia to white suckers.

The purpose of this study was to determine 96-h LC50 values for juvenile white suckers using the same dilution water and under similar conditions as tests conducted on the early life stages of this species (Reinbold and Pescitelli 1982).

MATERIALS AND METHODS

Test Organisms

Two 96-h flow-through toxicity tests were conducted on juvenile (Age 1) white suckers. The fish were obtained from northern Minnesota wild stock through Robinson's Live Bait, Inc., Genoa City, Wisconsin. They were transported to the INHS laboratory in 500 liters of filtered well water (14°C). Transport time was less than 4-1/2 hours. The transport water was gradually replaced with laboratory dilution water which had been adjusted to the proper temperature, and then the fish were placed into flow-through holding tanks. Ninety percent replacement time (Sprague 1973) for the holding tanks was never greater than ten hours during the acclimation period. Fish were held at the test temperature in the environmental chamber where the tests were conducted for at least 2 weeks prior to testing. During acclimation, the white suckers were fed a trout production diet (Rangen, Inc., Buhl, ID) two times a day, equalling approximately 3-4 percent of body weight. During acclimation, the photoperiod was increased by 15-minute increments every 2-3 days from 15 to 16 h. Mean size (and range) of white suckers used in the test was 92 (71-119) mm and 6.3 (2.6-13.2) g.

Dilution Water

Dilution water was taken from municipal wells at depths of 230 to 370 feet in the Mahomet-Teays aquafer near Champaign-Urbana, Illinois. The water was passed through two in-line charcoal filters to remove chlorine and through an ultraviolet sterilizer to eliminate microorganisms. It was then delivered through PVC pipes to a 340-liter stainless steel holding tank. Sodium thiosulfate was metered into the tank to remove any trace of chlorine which might remain after filtration.

A dilute solution of hydrochloric acid was also metered into the tank when necessary to adjust the pH to levels similar to those in the white sucker chronic test. Characteristics of the dilution water are listed in Table 1.

The dilution water was aerated in the holding tank. The water temperature was controlled with a portable cooling unit (Blue M Electric Company), and the holding tank was surrounded with fiberglass and foam insulation to maintain the desired temperature.

Exposure Systems

For each test the dilution water was pumped through PVC pipes from the holding tank to a 0.5-liter proportional diluter, modified from Mount and Brungs (1967) and Lemke, Brungs and Halligan (1978). Five concentrations and a control were delivered through mixing chambers to two replicate aquaria. In the first white sucker test, a logarithmic series of concentrations were used. For the second test, a 0.75 dilution series was used to narrow the concentration range. The flow rate to each test chamber was 0.25 liter every 4 minutes during both tests. The test chambers used for the fish were constructed of glass and silicone sealant. Each aquarium measured 30 x 40 x 20 cm with an overflow outlet at a height of 25 cm and contained a volume of 20 liters. Test aquaria were placed in a stratified random arrangement. The proportional diluter and the test chambers were placed inside a walk-in environmental chamber (2.2 m high x 2.75 m long x 2.2 m wide) which was maintained at the desired test temperature.

All apparatus, including holding tank and diluter, were cleaned prior to each test. Test chambers and other equipment coming in contact with test organisms were sterilized with hypochlorite before each test.

Reagent grade ammonium chloride was used as the toxicant. Stock

Table 1. Chemical characteristics of the dilution water.[a]

Al	<0.056
As	0.067
Ba	0.001
Ca	11.6
Cd	<0.020
Co	<0.004
Cr	<0.025
Cu	<0.005
Fe	<0.030
Hg	<0.00007
Mg	10.3
Ni	<0.017
P	<0.183
Pb	<0.036
Se	<0.061
Si	3.02
Zn	0.020
Soluble orthophosphate	0.01
Total dissolved ionizable solids	178
Total organic carbon	3.0
Specific conductance (mhos/cm at 25°C)	237
Total residue	142
Chloride	14.7
Sulfate	1.99
Fluoride	1.0

[a]All values are in mg/liter unless otherwise stated.

solutions were prepared in glass distilled water and delivered to diluters from a Mariotte bottle. The pH of the stock solution was adjusted to that of the control chambers with sodium hydroxide solution.

Photoperiod was automatically controlled for all tests using a combination of incandescent and fluorescent (including wide spectrum Durotest "Vita Lite") light bulbs. For both tests a 16-h photoperiod was maintained, including a 30-minute gradual brightening and dimming to simulate dawn and dusk.

Analytical Procedures

Water quality parameters were measured by using standard methods (American Public Health Association et al. 1976, U.S. Environmental Protection Agency 1979). Water samples were taken from the center of each test chamber.

During each test, simultaneous measurements of total ammonia nitrogen, pH, and temperature were made at least once each day during the first 4 days of the test. Total ammonia nitrogen concentrations were determined by the phenate method (American Public Health Association et al. 1976) using a standard curve prepared by linear regression. Colorimetric measurements were made with a Coleman 124D double beam spectrophotometer. Un-ionized ammonia concentrations were determined from total ammonia nitrogen, pH, and temperature using the tables of Thurston et al. (1979). The pH in each test chamber was determined at least twice daily with an Orion 701A digital pH meter. Dissolved oxygen was measured with an oxygen specific electrode calibrated to titration accuracy (Altex 0260 oxygen analyzer by Beckman). Measurements were made at least three times per week.

Hardness, nitrate nitrogen, nitrite nitrogen and soluble orthophosphate were determined using a Technicon Autoanalyzer (U.S.

Environmental Protection Agency 1979). Other water quality parameters such as alkalinity, conductivity, and TOC were determined according to analytical procedures described in American Public Health Association et al. (1976). Analyses of metals in the dilution water were performed by induction-coupled argon plasma spectrometry (American Society for Testing and Materials 1980).

Test Procedures

The methodology for the tests generally followed that recommended in Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (American Society for Testing and Materials 1980) and Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (U.S. Environmental Protection Agency 1975). The test organisms were distributed into the test chambers one-at-a-time and were acclimated to the test chambers for 2 days while the diluter operated without toxicant addition. The test was initiated when toxicant addition was started.

Mortality was recorded after 1, 3, 6, 12 and 24 hours and at least daily thereafter to the end of the test. No mortality occurred in control tanks. Death was determined by absence of gill movement and the lack of response to gentle prodding. Total length and weight were measured individually as mortality occurred or at test termination. Fish were not fed during the tests.

The 96-h median lethal concentrations (LC50) and their 95 percent confidence intervals were calculated from the un-ionized ammonia-nitrogen values in the test chambers by using the Trimmed Spearman-Karber method (Hamilton, Russo, and Thurston 1977).

The 96-h LC50 values and their 95 percent confidence intervals are reported in Table 11. Mean temperature and the range of pH values in all test chambers for each test are listed because of the importance of these variables in the equilibrium of ammonia in water, and mean values of other selected characteristics of the test water are also reported.

Mortality data, including the approximate time death occurred, are given in Appendix A. The 96-h LC50 values for the two tests were 1.15 and 1.11 mg/liter of un-ionized ammonia nitrogen. These LC50 values fall within ranges which have been reported for bluegills (Roseboom and Richey 1977) and for fathead minnows (Thurston et al. 1981). Rainbow trout are more sensitive (Thurston et al. 1981), and channel catfish are less sensitive (Roseboom and Richey 1977, Colt and Tchobanoglous 1978).

In an earlier study of the effects of ammonia on early life stages of the white sucker, using the same dilution water as in the acute toxicity tests reported here, a reduction in time to swim-up and a significant reduction in growth of the fry were found at a concentration of 0.06 mg/liter of un-ionized ammonia nitrogen.

Table 2. Acute toxicity of ammonia to white sucker. Conditions in test aquaria and 96-h LC50.

	Test I	Test II
Temperature[a] (°C)	15.0 (14.7-15.3)	15.4 (15.1-15.8)
pH	8.07 - 8.26	8.00 - 8.28
Dissolved oxygen[a] (% saturation)	93 (89-96)	88 (82-97)
Hardness (as $CaCO_3$)	45 (36-53)	50 (25-76)
Total alkalinity (mg/l as $CaCO_3$)	85 (63-103)	117 (104-134)
Nitrate nitrogen (mg/l)	<0.02	<0.02
Nitrite nitrogen (mg/l)	<0.01	<0.01
96-h LC50 with 95% CL[b] (mg/l un-ionized ammonia-N)	1.15 (0.95-1.38)	1.11 (1.01-1.22)

[a]Values reported are mean values of all measurements in all tanks with ranges in parentheses.
[b]Confidence level.

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Appendix A. Acute toxicity of ammonia to white sucker: time/percent mortality observations.

Time (hours)	Tank numbers											
	5	7	1	9	4	12	2	8	3	10	6	11
						Test I						
NH_3-N,mg/l	2.76	2.83	2.00	2.06	1.16	1.16	0.69	0.73	0.42	0.42	controls	
24	100	100	100	100	20	50	20	0	0	10	0	0
48	100	100	100	100	20	50	30	0	0	10	0	0
72	100	100	100	100	30	50	30	0	0	10	0	0
96	100	100	100	100	30	50	30	10	10	10	0	0
						Test II						
NH_3-N,mg/l	1.86	1.81	1.34	1.16	0.83	0.86	0.66	0.77	0.55	0.59	controls	
24	80	90	80	20	10	0	0	0	0	0	0	0
48	80	100	80	30	20	0	0	0	0	0	0	0
72	90	100	80	40	20	10	0	0	0	0	0	0
96	100	100	80	60	20	10	0	0	0	0	0	0